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Divisions of Carbohydrate Chemistry, Chemical History,
Medicinal Chemistry and Organic Chemistry

Implications of Sugar Ring Conformations in Drug Design: A Symposium in Memory of Muttaiya Sundaralingam

March 26, 2006

Symposium Organizers:

Joseph J. Barchi, Jr

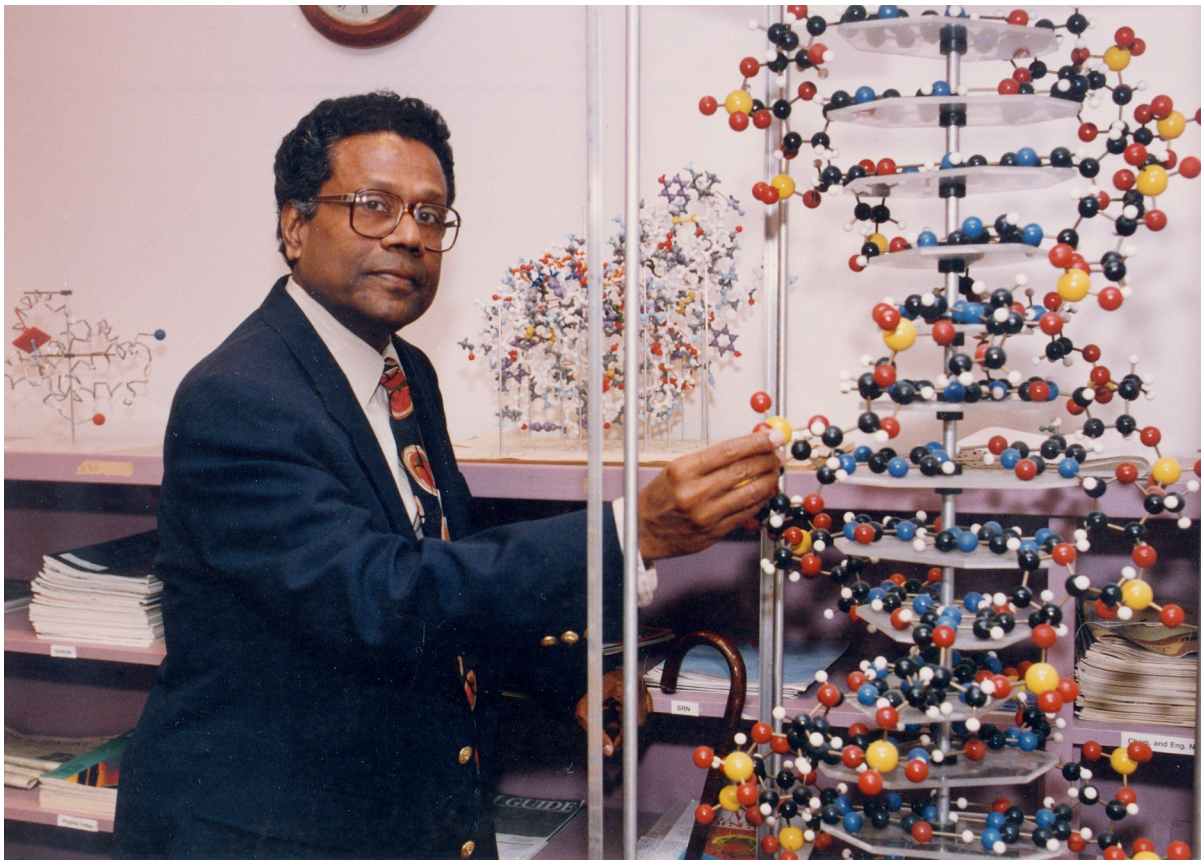
Laboratory for Medicinal Chemistry,
National Cancer Institute-Frederick

Todd L. Lowary

Department of Chemistry and The Alberta Ingenuity Centre for
Carbohydrate Science, The University of Alberta

Muttaiya Sundaralingam

September 21, 1931–December 26, 2004



Muttaiya Sundaralingam was born in Taiping, Malaysia on September 21, 1931 into a family of twelve children. He received his primary and secondary education in both Malaysia and Sri Lanka and, despite family pressure to become a teacher of the Tamil language, obtained his B.Sc. in Chemistry from the University of Sri Lanka-Colombo in 1956. Following his matriculation, he spent two years as an assistant lecturer in the Department of Chemistry at his *alma mater*.

In 1958, at the age of 26, Sunda, as he was known by his colleagues and friends, traveled by steamship to North America to do graduate work at the University of Pittsburgh. At Pittsburgh, he worked under the supervision of the legendary X-ray crystallographer George A. Jeffrey. After three years in the Jeffrey group, Sunda earned

his Ph.D. and in 1962 moved to The University of Washington in Seattle, where he was first a postdoctoral fellow and then a research associate with Lyle Jensen. It was in the Jensen group where Sunda was first exposed to the field of nucleic acid crystallography, through the accurate determination of the structure of cytidine-3′ monophosphate.

Following a short period at the Children's Cancer Research Foundation and Harvard Medical School in Boston, Sunda, in 1966, took a position as an Associate Professor in the Department of Chemistry at Case Western Reserve University in Cleveland. At Case Western, he started his studies into nucleic acid conformation by solving the X-ray structures of a number of nucleosides and nucleotides leading to a highly cited paper in *Biopolymers* (**1969**, *7*, 821–860), which laid the foundation for the conformational analysis of nucleotides and nucleic acids. The conformational preferences proposed in this paper have stood the test of time and remain valid within the huge number of nucleic acid crystal structures determined to date.

In 1969, Sunda moved to the University of Wisconsin, where he was a member of the Department of Biochemistry as the Steenbock Professor of Biomolecular Structure and Chairman of the Biophysics Ph.D. program. The bulk of Sunda's professional career was in Wisconsin and among his contributions from this period were crystallographic studies of tRNA. In addition, during his time at Wisconsin he carried out, with the collaboration of Cornelis Altona, detailed investigations on the conformation of the furanose ring in nucleic acids. This work led to the publication of a seminal paper (*J. Am. Chem. Soc*, **1972**, *94*, 8205–8212) in which the concept of pseudorotation was applied to the furanose ring. This paper remains a classic in the field and the two-state model proposed in this paper is still widely used in the conformational analysis of molecules containing five-membered rings.

After 21 years at Wisconsin, Sunda was offered and accepted the position of Ohio Regent's Eminent Scholar in Macromolecular

Structure in the Department of Chemistry at The Ohio State University. His group in Columbus continued to solve important nucleic acid structures and among his many contributions from this period was work demonstrating that distamycin forms a side-by-side complex in the minor groove of nucleic acid helices. In addition, the large number of crystal structures of DNA, RNA and RNA-DNA hybrid complexes solved by his group in Columbus provided critical insights into nucleic acid structure and also the role of C-H···O hydrogen bonds in structural biology. During his time at Ohio State he also ventured into the field of protein crystallography, working with Professor Ming-Daw Tsai on the structure of phospholipase A2.

Among Sunda's many awards was a Guggenheim Fellowship (1975–1976), which he spent in Oxford and an Alumni Award and Distinguished achievement award from the University of Pittsburgh (1986). Perhaps even more telling of his stature in the field was the more than 30 years of continuous funding he received from the National Institutes of Health.

Sunda formally retired from Ohio State in 2002, but remained active, publishing papers and collaborating with colleagues. In December 2004, he returned to his native Sri Lanka for a vacation where, on December 26, he and his wife Indrani were killed in the catastrophic tsunami that struck Asia. With his death, the world has lost one of the true pioneers in conformational analysis. His seminal work in the field of nucleic acid structure laid the critical groundwork for investigations that continue to this date and a number of these areas are highlighted in this symposium.

Joseph J. Barchi, Jr.
Todd L. Lowary
March 2006

Symposium Schedule

Session 1

9:00 AM Introductory Remarks

9:10 AM The pseudorotational cycle as a tool for drug design and discovery
Victor E. Marquez

9:50 AM Pucker determination in five-membered rings from residual dipolar couplings
Darón I. Freedberg, Scott E. Norris

10:30 AM Intermission

10:40 AM RNA interference and chemical modifications: Conformational and stereochemical aspects
Muthiah Manoharan

11:20 AM Arabinose-modified siRNAs ("siANA & siFANA")
Masad José Damha, Jonathan Watts, Nicolay Ferrari, Tom Dowler, Denis Bergeron, Anna-Lisa Tedeschi, Luc Paquet, Paolo Renzi

12:00 PM Lunch Break

Symposium Schedule

Session 2

2:00 PM: Introductory Remarks

2:05 PM: Evidences for the electrostatic promoted modulation of the chemical reactivity of nucleobases and phosphates in the single-stranded RNA
Jyoti Chattopadhyaya

2:45 PM: Covalent restriction of the nucleoside pentofuranose moiety: Bicyclic nucleosides as nucleic acid building blocks
Poul Nielsen

3:25 PM Intermission

3:35 PM Synthesis and conformational analysis of 2'-fluoro-5-methyl-4'-thioarabinouridine (4'S-FMAU)
Jonathan K. Watts, Kashinath Sadalapure, Niloufar Choubdar, Masad J. Damha, **B. Mario Pinto**

4:15 PM Hydroxyl group ionization and anomerization of aldofuranose rings
Anthony S. Serianni, Ian Carmichael

4:55 PM Concluding Remarks

Abstracts and Speaker Biographies

The pseudorotational cycle as a tool for drug design and discovery

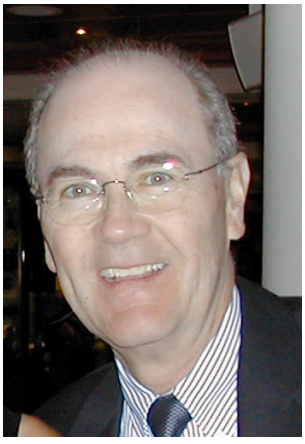
Victor E. Marquez
Laboratory of Medicinal Chemistry, CCR, NCI-F, NIH, NCI-Frederick, Frederick, MD 21702.

9:10 AM

Abstract:

Nucleosides behave as perfect molecular chameleons with an extraordinary ability to adapt their shape to achieve optimal catalytic efficiency when binding to target enzymes. These enzymes could be anabolic or catabolic, and for nucleoside-based drugs, the final therapeutic outcome will be determined by the ability of these molecules to bind effectively, or to avoid completely, interacting with certain enzymes. It has only been in the last two decades that the concept of pseudorotation, first proposed by Altona and Sundaralingam in 1972, has been fully embraced by the research community as a tool to describe the conformation of nucleosides. This presentation will provide specific examples where guided by the concept of pseudorotation a rigid bicyclo[3.1.0]hexane pseudosugar scaffold has been used to design drugs effectively locked in one of the two rapidly equilibrating North and South conformations of standard nucleosides.

Biography:



Dr. Marquez received his Ph.D. in medicinal chemistry from the University of Michigan in 1970. After 1 year of postdoctoral training at the National Cancer Institute (NCI), he worked in private industry for 5 years in Venezuela. He rejoined the NCI in 1977 as a visiting scientist and was awarded tenure in 1987 after becoming a naturalized citizen. His main research interests are nucleoside chemistry and synthetic organic chemistry as tools for the rational design of antitumor and antiviral agents. Dr. Marquez has authored or coauthored more than 280 publications and has received 25 U.S. patents.

Pucker determination in five-membered rings from residual dipolar couplings

Darón I. Freedberg
Office of Vaccines Research and Review, CBER/FDA, 29 Lincoln Drive, Building 29, Room 115, Bethesda, MD 20892

9:50 AM

Abstract:

RDCs (residual dipolar couplings) can be used to determine carbohydrate structure because they yield relative internuclear vector orientations. We determine five-membered carbohydrate ring pucker from a combination of $^{1}H–^{13}C$ one-bond, $^{1}H–^{13}C$ long-range, $^{13}C–^{13}C$ one-bond, and $^{1}H–^{1}H$ RDCs which fit the best structural model. Treating the five-membered ring as an isolated residue, 20 structures were generated, each with a different pucker and fit to RDCs. Best fits were found without NOEs or empirically parameterized Karplus curves. The pucker phase of the fructofuranosyl ring is between 20° and 70°, in agreement with previous studies. Furthermore, the analysis implies more than one stable pucker phase and rapid ring interconversion in this range. Thus, RDCs alone can reveal multiple puckers. We are currently applying the method to analyze the conformations of arabinofuranose rings from *Mycobacterium tuberculosis*, and to the deoxyribose ring of nucleosides in DNA lesions.

Biography:

Darón Freedberg received an A.B. degree from UCSD, where he worked under the tutelage of Jay Siegel focusing on stereo-chemistry and stereodynamics. He went on to earn his Ph.D. from UCLA, where he studied conformational isotope effects by NMR, under the direction of Frank A. L. Anet. During his post-doctoral studies Darón focused on using multi-dimensional, high-resolution heteronuclear NMR to understand how dynamics and structure of HIV protease/ inhibitor complexes influence binding and enzyme activity. His current research interests in carbohydrate structure and dynamics combine stereochemistry, conformational analysis, and cutting-edge NMR techniques to develop carbohydrate structure-function relationships.

RNA interference and chemical modifications: Conformational and stereochemical aspects

Muthiah Manoharan
Drug Discovery, Alnylam Pharmaceuticals, 300 Third Street,
Cambridge, MA 02142

10:40 AM

Abstract:

A critical requirement for achieving safe and efficacious RNAi therapeutics is introduction of "drug-like" properties, such as stability, cellular delivery, and tissue bioavailability, into synthetic siRNAs to improve in vivo pharmacological properties. Recently we modified potential endo- and exonuclase cleavage sites by appropriate sugar and backbone modifications and improved in vivo serum and tissue half live of siRNA duplexes. These chemical changes enhanced in vivo stability and efficacy which led to potent siRNA compounds. These results represent a significant advance in the development of siRNA therapeutics via appropriate chemical modifications and could be explained from the stereochemical and conformational features of chemically modified nucleotides.

Biography:

Muthiah Manoharan, Ph.D., is Vice President, Drug Discovery, Alnylam Pharmaceuticals. Dr. Manoharan was the former Executive Director of Medicinal Chemistry at Isis Pharmaceuticals, Inc., a leading biotechnology company focused on nucleic acid-based therapeutics where he had a 12-year tenure. With a distinguished career as a world-leading oligonucleotide chemist, Dr. Manoharan is the author of over 125 publications and over 100 abstracts, as well as the inventor on 100 issued U.S. patents. Prior to Isis Pharmaceuticals, he earned his Ph.D. in chemistry at the University of North Carolina-Chapel Hill working with Professor Ernest Eliel and conducted post-doctoral work Professor John A. Gerlt at Yale University and at the University of Maryland.

Arabinose-modified siRNAs ("siANA & siFANA")

Masad José Damha
Department of Chemistry, McGill University, 801 Sherbrooke Street West, Montreal, QC H3A2K6, Canada

11:20 AM

Abstract:

We present the physicochemical and biological properties of small interfering RNA duplexes (siRNA) containing arabinose modified nucleotide (ANA and FANA), as well as small interfering 2'-deoxy-2'-fluoroarabinonucleic acid(FANA):RNA hybrids for the downregulation of gene expression.

Biography:

Dr. Damha was born in and raised in Managua, Nicaragua. He received a B.Sc. ('83) and Ph.D. ('88) in chemistry from McGill University, the latter under the supervision of Prof. K. K. Ogilvie working on nucleic acid synthesis. Damha was awarded a NSERC postdoctoral fellowship in 1987, but declined the honor in favor of an Assistant Professorship at the University of Toronto's Erindale College. In 1992, he returned to his Alma Mater, where as James McGill Professor of Chemistry, he is involved in the synthesis and rational design of oligonucleotide analogues with antitumor and antiviral activity. Dr. Damha has authored or coauthored more than 100 publications, and has received 6 patents worldwide.

Evidences for the electrostatic promoted modulation of the chemical reactivity of nucleobases and phosphates in the single-stranded RNA

Jyoti Chattopadhyaya
Department of Bioorganic Chemistry, Box 581, BMC, University of Uppsala, Husargatan 3, S-75123 Uppsala, Sweden

2:05 PM

Abstract:

We here report that the neighboring bases in ssRNA are electronically coupled as a result of variable electrostatic modulation, depending upon the sequence-context, giving variable pKa perturbations as a result of variable pseudoaromatic characters. The net result of this electrostatic cross-talk between two neighboring aglycones as a result of base-base stacking is creation of a unique set of aglycones in an oligo or polynucleotide, whose physico-chemical properties are completely dependent upon the nearest neighbor electrostatic interactions. This has considerable implication in the specific ligand binding ability, aptamer recognition, RNA catalysis, and most probably in codon-anticodon interaction. Physical as well as chemical reactivity studies will also be presented showing that the electronic properties (charges), and consequently the electrophilic character of the internucleotidic phosphates of the internucleotidic phosphodiester bonds in the single-stranded RNAs, are indeed dissimilar in a sequence-specific manner because of their non-identical microenvironments, in contrast with the corresponding isosequential ssDNAs.

Biography:

Prof Chattopadhyaya received his Ph.D. in Organic chemistry from the National Chemical Laboratory, Poona, India in 1974. After 5 years of postdoctoral training in the area of Nucleoside and nucleotide chemistry at the King's College University of London, UK with Prof C.B. Reese, he joined Uppsala University as an Assistant Professor. He became a tenured Associate Professor in 1982. In 1985 he was appointed to the Chair of Bioorganic Chemistry in Uppsala University, and currently is a holder of the Chair. He has worked in last 30 years in the interface of Chemical synthesis, physical chemistry and structure of DNA and RNA to understand their biological reactivities. His major contribution lies in the elucidation of stereoelectronic principles governing the dynamic structural preferences of nucleosides, nucleotides and oligonucleiotides. His recent work includes stacking and extended genetic code governed by nearest-neighbor interactions, and the demonstration of the non-equivalency of internucleotidic phosphates based on the nature of sequence-context. Dr. Chattopadhyaya has authored or coauthored more than 365 publications and has received 3 U.S. patents (Homepage: www.boc.uu.se).

Covalent restriction of the nucleoside pentofuranose moiety: Bicyclic nucleosides as nucleic acid building blocks

Poul Nielsen
Department of Chemistry, University of Southern Denmark, Odense, Campusvej 55, 5250 Odense M, Denmark

2:45 PM

Abstract:

The ultimate conformational restriction of the nucleoside pentofuranose ring has been introduced by with LNA (locked nucleic acids). The LNA monomers are locked in a North-type conformation by an additional oxymethylene bridge connecting the 2L- and the 4L-positions. Even a few LNA-monomers can stabilise nucleic acid duplexes significantly. Other bicyclic nucleosides varying the strength and positioning of the conformational restriction (e.g. 2L-3L, 2L-4L, 3L-4L bridges), the resulting conformation (North, South and East-type conformational mimics) or the stereochemistry of the pentofuranose moieties has been concerned. Our recent results with bicyclic nucleosides will be presented. The stereoisomers of LNA, α-D-LNA and β-L-LNA, have shown a strong tendency to form parallel nucleic acid duplex with target RNA sequences. Also an East-type mimicking α-D-configured bicyclic nucleoside has been shown to improve the stability of parallel nucleic acid duplexes. Other bicyclic nucleosides prepared by a ring-closing metathesis (RCM) based strategy will be presented.

Biography:

Poul Nielsen was born in Denmark and received a M.Sc. and a Ph.D., 1994 and 1998, respectively, from Odense University, Denmark with Professor Jesper Wengel. He spent seven months in 1997 as a guest researcher with Professor S. M. Roberts, University of Liverpool, UK. In 1998 he started his independent career as assistant professor and from 2001 as associate professor in organic chemistry at the University of Southern Denmark. His main research interests are synthetic chemistry within nucleic acid chemical biology and nanobio-technology; Synthesis of conformationally restricted and derivatised nucleosides and nucleotides; Metathesis reactions on nucleic acid derivatives; Click chemistry; Double-coding nucleic acids; Molecular recognition of secondary RNA structures; Nucleotide-antibiotic conjugates.

Synthesis and conformational analysis of 2'-fluoro-5-methyl-4'-thioarabinouridine (4'S-FMAU)

B. Mario Pinto
Department of Chemistry, Simon Fraser University, 3195 Strand Hall, 8888 University Drive, Burnaby, BC V5A 1S6, Canada

3:35 PM

Abstract:

An improved synthesis of 2'-deoxy-2'-fluoro-5-methyl-4'-thioarabinouridine (4'S-FMAU) is described. Participation of the 3'-O-benzoyl protecting group in the thiosugar precursor influenced the stereochemistry of the N-glycosylation reaction in nonpolar solvents, permitting a higher <=± ratio than previously observed for similar Lewis acid-catalyzed glycosylations. Conformational analysis of the nucleoside using $^3J_{HH}$ and $^3J_{HF}$ NMR coupling constants together with the PSEUROT program showed that it adopted a predominantly northern conformation, in contrast to 2'-deoxy-2'-fluoro-5-methylarabinouridine (FMAU), whose PSEUROT conformational analysis is presented here for the first time and which showed a dominantly southeast conformation. The sharp conformational switch attained by replacing the ring heteroatom is attributed to a decrease in relevant steric and stereoelectronic effects.

Biography:

Dr. Pinto was born in Colombo, Sri Lanka and received his B.Sc. degree in Chemistry and Ph.D. from Queen's University. Dr. Pinto served as Chair of the Chemistry Department from 1999-2004, and is currently Vice-President, Research at Simon Fraser University. Dr. Pinto received the 1992 Horace S. Isbell Award of the American Chemical Society, the 1993 Merck Frosst Award of the Canadian Society for Chemistry (CSC), the 2002 Bernard Belleau Award of the CSC, and the 2005 BC Innovation Council Frontiers in Research Award. He is a Fellow of the Chemical Institute of Canada, and was elected to the Academy of Sciences of the Royal Society of Canada in 2003. Dr. Pinto is a pioneer in the field of chemical biology having developed novel NMR/molecular modeling protocols for protein structure determination and the study of ligand topographies essential for drug and vaccine design. He was recently awarded a patent for his breakthrough on the effect of glycosidase inhibitors as novel therapeutic agents for Type 2 diabetes, which has proven effective in lowering blood glucose levels in rats. He is founder of the company, Mimos Therapeutics, Inc.

Hydroxyl group ionization and anomerization of aldofuranose rings

Anthony S. Serianni
Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556-5670

4:15 PM

Abstract:

Theoretical studies of aldofuranose rings using quantum mechanical methods have been used to study the effects of molecular structure on NMR parameters such as spin-spin coupling constants. The inherent flexibility of these rings (pseudorotation) renders them valuable in investigations requiring systematic and continuous alteration of the relative positions of ring substituents without the need to break bonds. Information derived from these systems over the past 15 years has helped interpret results from recent and related studies of aldopyranosyl rings. In the present work, we investigated, using DFT methods, the effect of orientation (quasi-axial vs quasi-equatorial) on the acid dissociation constants of hydroxyl groups of furanosyl rings. We were interested in this problem due to the proposed role of the ribonucleotide 2'-OH group in the mechanism of ribozyme catalysis. These studies also yielded new insights into the mechanism of furanose anomerization, specifically whether discrete conformations of furanose rings are more prone to ring-opening (and presumably ring-closing) than others.

Biography:

Anthony Serianni was born in Chestnut Hill, Pennsylvania and received his B.S. in biochemistry from Albright College, Reading, PA. He pursued graduate studies in the Department of Biochemistry at Michigan State University, earning his Ph.D. in 1980 under the guidance of Professor Robert Barker. He moved to Cornell University, Section of Biochemistry, for postdoctoral training, and in 1982, joined the faculty in the Department of Chemistry and Biochemistry at the University of Notre Dame, where he is currently Professor of Chemistry and Biochemistry. In 1982, he co-founded Omicron Biochemicals, Inc., a biotech company specializing in the synthesis of stable isotopically labeled carbohydrates, nucleosides and their derivatives. Now in its 24th year of operation, Omicron is located in South Bend, Indiana and supplies a wide range of non-GMP and GMP products to an international group of clients. Some of Professor Serianni's research interests include methods development for site-specific labeling of carbohydrates, conformational studies by NMR of simple and complex carbohydrates related to the *N*-glycans of human glycoproteins, applications of molecular orbital theory to aid in the interpretation of NMR parameters, and structure-function studies of non-enzymic protein glycation. Dr. Serianni has co-authored over 100 peer-reviewed research papers, has been a recipient of the Horace S. Isbell Award sponsored by the Division of Carbohydrate Chemistry, American Chemical Society, and recently received the John Boezi Memorial Alumnus Award from Michigan State University.

Professor Muttaiya and Mrs. Indrani Sundaralingam Memorial Fund

Established in 2005, the mission of the fund is to:

- Help those in need in Sri Lanka (the orphans, the poor and the tsunami victims).
- Provide scholarships to students in both the United States and Sri Lanka at the undergraduate and graduate level.
- Provide assistance for any future disasters in the United States or Sri Lanka.

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